Some Remarks on Enzymes

by

C. A. PEKELHARING.

The remarks put forward in this paper on the comprehensive subject of the action and the nature of enzymes were intended as a guide for a lecture I was prevented from delivering. Now that I am no longer in a position to participate in experimental inquiry I feel inclined to publish them as a concise, and, it must be added, as a very incomplete compendium of the conceptions I derived from the work of others and partly also from my personal investigations.

Already in the very first stage of the knowledge of chemical changes in the animal body, observers were struck with the remarkable phenomenon that various conversions are brought about much more easily in the body than out of it. It became conspicuous especially with regard to the food taken into the alimentary canal. Solid food, such as meat e. g., when reappearing from the stomach a short time after it had been taken up by it, proved to have been changed into a thin pap, just as happens outside the body through prolonged boiling in water. Initially the body temperature was suspected to be answerable for it.

The first who made opposition to this conception was Johannes Baptist Van Helmont (1577—1644), the

man who after Paracelsus laid the foundations of Physiological Chemistry. He set forth at large that this view is completely at variance with practical experience [1]).

It is, he states on page 192, a deceptive metaphor of the School, when it identifies digestion with boiling, "concoctio" with a poetical licence borrowed from the peasant mind. The mild heat of the animal body is by far incapable to answer for the solution of the foodstuffs in the stomach. Besides also in the cold-blooded fishes the food is digested in the stomach. If digestion were brought about by heat, a higher temperature would accelerate digestion; now with fever patients this it not the case at all. We had better, Van Helmont says (p. 195), turn aside from this folly. If, by chance, anything of the food sticks in the hollows of the teeth, it is not digested, but it soon putrefies, emitting a horrible stink.

He is surprised to see that even Paracelsus was betrayed into giving credence to the digestive power of heat and that he remained unconscious of the existence of the gastric ferment. For the foods are dissolved by a ferment with acid properties, formed in situ. However this is not a ferment on account of its being acid, but because it is a ferment, for vinegar or red currants, though sour and acrid, do not on that score ferment (p. 111).

Among the followers of the School there is no conception so simple as that about ferment, but at the same time none is more useful, thus Van Helmont begins the chapter "Imago fermenti". Things do not undergo a change, he continues, or a conversion through an imaginary propensity of the substance itself, but only through the action of a ferment. This is the case not only with the contents of the stomach, but also with that of the small and the large intestine; likewise the food is changed in

---

1) Opera Omnia, Francofurti, apud J. J. Erythropilus 1682.

the blood into the vital slimy substance that constitutes the essential food of the body. This food, Van Helmont proceeds (p. 108), sprinkles the separate parts of the body like dew, as it in often said, but I believe that it is prepared in the several small kitchens of those parts.

It appears then that as early as three centuries ago the Flemish physiologist advocated the now universally received theory that not only in the alimentary canal but also in the blood and in the organs of the body highly significant changes are evolved by ferments, or, as we now more correctly say, by enzymes.

But Van Helmont's time was not nearly ripe yet for the further development and substantiation of an idea of such pregnant significance, which could bear fruit only when the knowledge of nature should have been considerably increased. Not before the middle of the 19th century was science so far advanced that an inquiry into the appearance and the nature of enzymes could be undertaken with greater success. Thenceforth numerous examinations of vegetable as well as animal organisms, spread some light, far from brilliant, it is true, but it clarified our ideas in many ways and opened up fresh paths of inquiry.

In 1876 Kühne suggested to designate the substances first pointed out by Van Helmont, as enzymes and to dinstinguish them from ferments [1]). What of old was called "fermentation" is caused by micro-organisms, as Pasteur has demonstrated. When grapes are damaged, yeast-cells, living on the outside of the skin come in contact with the pulp. They develop in it, multiply, and convert the sugar of the grape juice into alcohol and carbonic acid. This action, then, depends on the living matter of the

[1]) Verh. d. Naturk. und Med. Ver. zu Heidelberg Bd. I N. F. 4 Febr. 1876.

yeast i. e. the ferment. But when the cell pellicles of the yeast are fissured, a fluid may be produced by crushing, or if the yeast has first been dried and subsequently rubbed down to powder, by maceration in water, which fluid, when mixed with a sugar solution also splits up the sugar and evolves alcohol and carbonic acid. This activity the fluid obtains from a substance called zymase, which indeed originates from the living matter of the yeast cells, but is unable to multiply, and cannot, therefore, be said to live.

To give another example, there are bacteria that, when growing up in a fluid containing ureum, can convert this substance into ammonium-carbonate. From these bacteria, that go by the name of ureum ferments, and also from several products of higher plants, from soya-beans e. g., a substance, called urease, can be extracted with water, that converts in the same manner ureum into ammonium carbonate.

Kühne's suggestion to differentiate these lifeless substances, active still without any connection with the cells, from the living ferments, by the name of "enzymes", tended to prevent confusion of terminology, and was readily accepted by biologists, whose field of research comprises living organisms as well as the substances produced by them. Afterwards, however, when Bredig discovered that colloid suspensions of some metals, as well as catalase, an enzyme present in the blood and many organs of the animal body, are capable of decomposing hydrogen peroxid into water and oxygen, he called these colloid metals inorganic ferments. In conjunction with him some inquirers, especially German, have abandoned the term enzymes, und adopted again the name of ferments in all cases. It can hardly be denied, that this will lead to fresh confusion in the nomenclature, which, indeed, is great enough already.

— In course of time a considerable number of enzymes have come to our knowledge. The substance, which, as observed by Van Helmont effects the digestion of foods in the acid gastric juice, and, as demonstrated much later, dissolves and splits up especially proteins, must be grouped under the proteolytic enzymes, which are largely represented in the animal body, and which also occur in the vegetable kingdom. Then there are enzymes, splitting up esters, particularly fats, also enzymes decomposing compound carbohydrates into more or less simple ones, and again others, generating in some way oxydation, while in all these groups a number of varieties are to be found. Far greater than the number of enzymes is the number of names by which they are designated. Duclaux has proposed to coin the name of an enzyme from that of the substance it can attack, by affixing the termination: "*ase*". Thus an enzyme forming glucose from maltose is called "maltase"; an enzyme splitting up lactose into galactose and glucose, "lactase"; a fat-splitting enzyme "lipase". But a number long known enzymes, such as ptyalin, pepsin, trypsin have retained their old-established names from sheer force of habit. Some are indicated now by one name, and now by another, as e. g. invertin, an enzyme that, when acting on canesugar changes the dextrorotatory power of the solution into a levorotatory power, and that afterwards was called by some researchers "invertase" or "saccharase" or "sukrase". Nor has Duclaux's principle always been applied correctly. Oxydases, for example, is the name given not to enzymes that attack oxygen or acids, but to those that cause oxydation.

A systematic and expressive nomenclature can be attained only when sufficient knowledge has been acquired of the nature and the composition of enzymes, which is not the case by for at present. What we know about

it is chiefly that enzymes are colloidal substances that they are inert or nearly so at a temperature of 0° C., that they gradually become more active at a higher temperature, until a certain optimum is reached which varies with the enzymes and that they are destroyed at a still higher temperature.

In establishing the presence of an enzyme researchers have ever employed the method of Van Helmont, who, after discovering that the digestion of the food in the stomach could no more be attributed to the acid than to the body heat, came to the conclusion that, besides the acid someting else must be produced by the stomach, viz. a "ferment".

Still, it must be granted that our knowledge of the actions of enzymes has increased considerably.

Whereas Van Helmont was quite ignorant of the composition of food, such as meat or bread, which he watched digesting, chemistry has unfolded to us its several constituents, and has enabled us to distinguish their properties and composition, as well as the decomposition products that can be obtained from them. Only then did it become possible for researchers to study minutely the way in which enzymes act on certain substances such as egg-white, starch etc.

— Of late years the advancement of physical chemistry has been highly favourable to the study of enzyme action, above all after the appearance of Van 't Hoff's works.

A remarkable fact, early discovered by researchers into enzyme action is that the quantity of a substance an enzyme can convert does not stand in a definite proportion to the quantity of the enzyme itself. When a solution of an acid is mixed with a solution of a base, acid and base both disappear from the solution while salt is being formed in a fixed proportion of weight. On the other hand even an unappreciably small quantity of an enzyme

is capable of converting large quantities of substances on which it can act. The enzyme does not disappear in the reaction and is ever able to convert fresh quantities of the original substance, unless it be destroyed, under unfavourable conditions, by the reaction or by the temperature of the fluid.

The fact discussed just now is, indeed, well known in chemistry also, and termed by Berzelius *"catalysis"*.

Proteins and starch, for example, may be decomposed it the same way as by the enzymes of the digestive organ, by boiling with mineral acids, though the acid itself does not undergo any change in the process.

Prolonged boiling in water with neutral reaction also causes, very slowly though, such a decomposition of albumin or starch. It appears, then, that the action of the acid consists in an increase of the reaction velocity.

It is the same with enzymes. Canesugar, e. g., dissolved in water and sheltered from any contact with lower organisms, undergoes no change worth mentioning, at normal roomtemperature, but is split up into glucose and fructose on prolonged boiling, very slowly though, while water is being taken up. This splitting is due to hydrogen-ions which are always present even in pure water, for when the amount of those ions in the solution is raised through the addition of any acid, the splitting is accelerated. At boiling temperature it is completed in a short time. It does not, therefore, seem an unjustifiable assumption that in some degree hydrolysis takes place also at room-temperature, also with neutral reaction; so slowly, however, that it takes a very long time to establish it. Now if to such a neutral sugar solution a small quantity of an extract of yeast is added, in which there is some invertin, or a little of an extract of the mucous membrane of the small intestine that contains a similar enzyme, a considerable portion of the sugar will be hydrolysed in a short time,

though the amount of hydrogen-ions has not undergone any change. This goes to show that the enzyme that is derived from yeast and displays intense action already at roomtemperature, or that originates from the intestinal mucous membrane, and acts better at body-temperature, leads to the same results as are obtained by applying acids and boiling temperature.

The same occurs with other enzymes, though some that belong to the same group do not carry the hydrolytic process so far as others. This is instanced by the proteolytic enzymes (proteases), secreted by the higher animals into the alimentary canal.

Proteins are split up, through prolonged boiling with hydrochloric acid or sulphuric acid, entirely into comparatively simple substances. By far the greater part of the hydrolytic products consist in amino-acids that are all derived from fatty acids, and several of which are still combined with cyclic groups. Now when protein is taken up as food it is first affected by the pepsin of the gastric juice, which with acid reaction splits up the protein, going scarcely farther, however, than the formation of albumoses, substances still retaining on the main the characteristics of protein and to be looked upon as large complexes of amino acids. Only very small quantities of free amino acids are demonstrable among the digestion products, evolved by pepsin from protein. The protein not digested by the stomach and the albumoses find in the intestine another enzyme, procured by the pancreas and called trypsin. Now the splitting proceeds. The trypsin liberates, with neutral or weak alkaline reaction, a number of amino acids from the protein and from the albumoses, but still, how long the action of the trypsin may be protracted, part of them is left behind in complexes, to which the name of "*peptids*" is given by E. Fischer. Now these also are split up hydrolytically

by "erepsin", an enzyme derived from the intestinal mucous membrane, so that finally, at the mild temperature of the animal body and with a nearly neutral reaction, all the animo acids have been set free from the protein, just as well as by boiling with mineral acids of rather strong concentration. The final stage has now been reached through the successive actions of three different, though all proteolytic, enzymes. The action of trypsin goes farthest, pepsin only starts the splitting, erepsin, on the other hand, which, with a few exceptions only, cannot attack genuine proteins but acts powerfully on albumoses an peptids, completes the work left undone by the trypsin.

Besides the temperature the amount of H- and OH-ions in the fluid also exerts a great influence upon the catalytic faculty of the enzymes. Generally the enzyme cannot stand a considerable excess of either. Pepsin is excepted, as its action is intense only with a weak, but still very distinctly acid reaction. Ptyalin, on the contrary, the enzyme of the saliva that resembles barley diastase and that converts starch into dextrin and maltose, acts best, as Ringer and Van Trigt[1]) have made out at body-temperature with an amount of H-ions only inappreciably larger than that of pure water at the same temperature. However, its activity is not checked altogether, when the reaction becomes slightly alkaline, in which case pepsin is completely destroyed in a few moments. As regards the resistance to H-ions trypsin slightly surpasses ptyalin. However it also is destroyed gradually after a sojourn at body-temperature in a fluid of alkaline reaction, which still admits of intense action.

Only for some enzymes do we possess very accurate data regarding the reaction at which they act most intensely

[1]) Onderz. Physiol. Lab. Utrecht 5e Reeks XIV p. 127. Zeitschr. f. physiol Chemie LXXXII S. 484.

and regarding the amount of shifting of the reaction they are capable to stand. The inquiry into this problem is difficult and takes much time; due care should also be taken that the change of reaction does not influence the enzyme alone, but also the substance attacked by the enzyme, not only by the alteration of the amount of H- and OH-ions brought about by the addition of an acid or a base; also the anion of the acid and probably also the cation of the base is of some significance for the enzyme action.

Some enzymes require for an action of some consequence the presence of neutral salts. Such is the case for instance with ptyalin, the enzyme of the saliva that attacks amylum. If saliva is dialysed against distilled water, it loses its action on starch, but regains it after the addition of a small amount of a chlorid, e. g, sodium- or potassium-chlorid. A small amount of alkaliphosphate may here be of some use, if proper care be taken that the reaction does not shift too much to the acid, or above all not to the alkaline side.

— In other cases it appeared that an enzyme required the co-operation of other substances, unknown as yet and provisionally termed *"co-enzymes"* — not a happely chosen word by the way. The name was introduced by Gabriel Bertrand in connection with his remarkable researches on the formation of Japanese lacquer from the Sap of the Rhus succedanea. Bertrand made out that lacquer was formed by an enzyme, which he called laccase, whose activity appeared to be excited by the addition of another substance, which, on further investigation proved most likely to be a manganese compound and is not at all to be considered as an enzyme.

Likewise it appeared that in the formation of alcohol from sugar by zymase, a phosphoric acid carbohydrate ester plays a part. Also this substance, that may be

separated from the zymase through dialysis and can stand boiling, cannot really be called a co-enzyme.

— In opposition to this the existence of *„anti-enzymes"* has been assumed. Judging from the name we should think it to imply counteracting enzymes. This is by no means the case. The name conveys nothing but an inhibition to the action of an enzyme in some sort of environment, without any ground for ascribing that inhibition to the influence of a definite group of substances, still less so to enzymes. Contrariwise, it has already appeared that the inhibition may be caused by substances and conditions of very different nature, while in one case the inhibiting influence will act on the enzyme itself and in the other on the substance attacked by the enzyme, i. e. on the subtrate, as it is generally termed, or on the "enzymotele" as Beyerinck has it.

It may be objected that it will not do to disapprove of the use of names such as co-enzymes and anti-enzymes, for substances, whose existence is only surmised on the basis of certain actions, and, at the same time, to speak of enzymes, whose nature we are equally ignorant of, and whose existence is assumed only on the ground of certain actions that have been observed. But in reasoning thus the fact is overlooked that this much at all events is known about enzymes: that they all are colloidal substances, that are destroyed at 65° C. or higher, and that they have catalytic power, which, on heating, increases gradually from the freezing point up to an optimum of temperature. It is these properties that justify us in considering enzymes as substances of a special kind, that must all be grouped together. The experimental inquiry into enzyme action lends support to this view.

They are catalysts and as such they are all of the same nature not only as regards hydrolysis but also with respect to synthesis of substances.

A number of substances is known that, dissolved in water and left standing, are slowly decomposed, but which decomposition when reaching a certain point, comes to a standstill. Also when such a reaction is accelerated by a catalyst, it is arrested at the same point. A simple illustration is an aqueous solution of acetic acid ethylester. After some time, which is considerably shortened on addition of a little hydrochloric acid, while water is taken up, part of the ester will be found to have been decomposed into acetic acid and ethylalcohol. Whether a catalyst is added or not, that does not matter, in either case the reaction is arrested as soon as certain ratio is reached between the amounts of acetic acid and alcohol on the one side and non-hydrolysed ester on the other. Obviously there now exists a state of equilibrium between the decomposition of ester and a synthesis of it from acid and alcohol. For, when starting with a solution containing no ester, but only acetic acid and ethylalcohol, dissolved in water, in amounts that correspond as to their number of molecules, ester is evolved, again under the influence of a catalyst with greater rapidity, until the ratio of acid, alcohol and ester is the same as before. When the point of balance is reached hydrolysis and synthesis are counterbalanced. Already many years ago Van 't Hoff has in his work on the phenomena of chemical equilibrium, advanced the hypothesis that, if enzymes are indeed to be considered as catalysts, there must be a way to detect synthesis as a result from enzymic action.

This was in fact found to be the case first by Croft Hill, who detected that, under the influence of maltase, the enzyme that splits up maltose into two molecules of glucose, a disaccharid is formed in a solution of pure glucose. It is true, that the evolved sugar appeared to be a mixture of maltose and its isomere, isomaltose, which is built up of two molecules of β-glucose, whereas

maltose is formed from two molecules of α-glucose, which conflicts with the hypothesis. For maltase does not attack isomaltose and on that account could not lead to an equilibrium between this sugar and glucose. The explanation may be found in the fact that, as Bayliss[1]) remarks, the enzyme that Croft Hill experimented with, was not maltase, purified as much as possible, but beer-yeast, which not infrequently contains besides maltase also emulsin, an enzyme that attacks all sorts of β-glucosids and consequently also isomaltose, so that at the same time isomaltose as well as maltose could be generated by the two enzymes.

— However, though for some time the significance of Croft Hill's experience was still a matter of dispute, further investigations yielded inequivocal instances of reversible action of enzymes. Bourquelot and his co-workers succeeded in preparing quite a series of alcohol-glucosids, the production being often as copious as is obtained in the chemical industry, by means of a β-glucoxydase from emulsin or by means of an α-glucoxydase from yeast, which tallies completely with the theory of chemical equilibrium[2]).

— Kastle and Loevenhart, Dietz and others showed that fatsplitting enzymes, lipases, also are capable of building up fat from fatty acids and glycerin. When we take pure fat, the hydrolysis proceeds until a certain ratio is reached of the quantity of the splitting products to the quantity of fat still intact. If in the outset the lipase acts upon the pure splitting products, fat is formed, till the same equilibrium is arrived at. It goes without saying that, inconsiderable anomalies will occur in the

---

[1]) "The Nature of Enzyme action". London 1914 p. 56.

[2]) Journ. de Pharm. et de Chimie, 7me Sér. Tome X p. 361, 393 Ann. dc Chimie 9me Série T. III p. 287.

experiments, but, as Bayliss has set forth, they may readily be attributed to various causes.

In the case of hydrolysis of cane-sugar under the influence of invertin, splitting proceeds so rapidly and so intensely that it seemed doubtful whether there was any question of an equilibrium reaction. To all appearance all the canesugar was hydrolysed to glucose and fructose. But Visser demonstrated by very minute experimentation, that about 1 % of canesugar always remained intact and on the other hand that in an invertin-solution with glucose and fructose in equal amounts the same quantity of canesugar was formed. The decomposition, indeed, proceeded much quicker, about 50 times, than the synthesis, but still the same equilibrium was attained in either case [1]).

— If, however, the splitting products are gradually thrown out of solution during the working of the enzyme, hydrolysis can prooced much farther than is compatible with the equilibrium of the original solution. As Sheridan Lea showed already many years ago, the splitting of starch by ptyalin, and of egg-white by trypsin, was to a great extent furthered by carryingout the digesting process in a dialyser, by which means the small molecules of the digestion products were removed from the dialyser directly they came free. In a similar way the digestion of the constituents of our food is promoted in the alimentary canal. The wall of the intestine resorbs the sugar that originates from the starch, the amino-acids that are derived from the proteins, the fatty acids and the glycerin resulting from the fat, so that equilibrium is out of the question since one scale of the balance is continually being deprived of the decomposition products.

— The sugar resorbed by the wall of the intestine, is

[1]) Zeitsch. f. Physik. Chemie Bd. LII S. 257.

transferred by the vena portae to the liver, where an enzyme, present in the liver-cells, builds up from it a carbohydrate, much more complicated, glycogen, that is lodged in the cells in the shape of little lumps and may be piled up in a larger quantity, according as more sugar is supplied. If, however the blood has become poor in sugar either through abstinency or through large use of sugar, the same enzyme can attack the store of glycogen and produce from it sugar which is directly carried off by the blood until a fresh supply is provided by the intestine.

— Fatty acids and glycerin are combined in the epithelium of the intestinal mucous membrane, by the lipase it contains. The fat, thus formed, is separated in the cells in small drops, that are gradually taken up by the chyliferous vessels and transferred to the blood. In this way the synthesis of fat can continue in the cells all the time the flow of fatty acids and glycerin from the hollow of the intestine is sustained. In the blood the fat is decomposed by the lipase, the hydrolytic products being removed by the cells of the body, to be consumed there or to be converted to fat again in the adipose tissue, which also contains lipase, and there to be preserved as in a storehouse.

With regard to the lipase of the pancreas it has been otherwise demonstrated that enzyme action is promoted by means, that prevent a state of equilibrium between fat on the one side and fatty acids on the other [1]). This lipase is all but insoluble in neutral water, as observed by Rosenheim, but, like most other enzymes, it readily dissolves in glycerin. Now when a glycerin-extract of the pancreas is highly diluted with water, the lipase is

---

[1]) Onderz. Physiol. Laborat. Utrecht 5e R. XIII p. 16 and Zeitschr. f. physiol. Chemie Bd. LXXX S. 355.

precipitated, so that it can in this way be separated from the other constituents, also from the other enzymes of the pancreas. Thus can be obtained a substance, that is all but free from electrolytes, that yields in glycerin a colloidal solution, keeping constant for a long time, and that does not hydrolyse protein and carbohydrates, but splits up fat in a very small measure. If, however a slight quantity of a limesalt is added, the action on fat is considerably intensified. This seemed to be due to the fact that, as soon as fatty acid is set free, it is fixed as an insoluble calcium soap. So the enzyme can keep on decomposing the fat without any synthetic counteraction. When the added salt was calcium chlorid, an acid was liberated that, according to the amount of H-ions in the fluid, was highly dissociated, and that in this case could only be hydrochlorid acid. When the action was increased by calcium carbonate, carbonic acid was evolved. Barium- and magnesiumsalts acted similarly. Also other electrolytes, sodiumsalts could be used, potassum-salts, however, only to a small degree, as could be expected, since potassium-soaps are more easily soluble in water than sodium-soaps.

— That some researchers failed to demonstrate the favourable influence of limesalts on the action of lipase, is most likely to be ascribed to the fact, that they did not use the purified, salt-free enzyme for their experiments, but extracts of the pancreas, or pancreas-juice, which, besides many other ingredients, also contain lime.

It is evident that it would be wrong to speak of co-enzymes here. Electrolytes such as limesalts do not act on the enzyme. This is borne out by the fact that they not only fail to further the synthesis of fat from fatty acid and glycerin under the influence of lipase but rather inhibit it, contrary to cholic acid salts, which promote both synthesis and hydrolysis.

The above warrants the conclusion, that enzymes must

indeed be considered as catalysts. They increase the rate of equilibrium-reactions, without altering the equilibrium itself. In case the decomposition proceeds farther than could have been expected in view of the original composition of the fluid, this is due to removal from the solution either of the decomposition products or of the matter evolved synthetically. If the equilibrium with the enzyme action is not attained, this may in many cases be attributed to destruction of the enzyme, that will easily occur especially in a somewhat alkaline fluid.

All this has been so well established for several enzymes, that the same view has been generally adopted also for other enzymes, even though no evidence has as yet been brought forward to support this view, in consequence of experimental difficulties.

This consideration however does not bear upon the conception to be formed about the mode of enzyme action. — Modern chemistry may have somewhat elucidated this problem, still not nearly enough to furnish a true insight into the matter.

All the enzymes we know, are colloidal substances. So, if a solution of an enzyme in water is spoken of, it should be borne in mind that, contrary to what occurs in a true solution, the separate molecules do not, dissociated or otherwise, move about free in the water, but that the molecules of the enzyme, associated in groups, are suspended in the water. Those groups may then be soaked with water and belong to the hydrophilous or emulsion-colloids, but this is not the case with all enzymes.

Bourquelot found that emulsin prepared from almonds, acts very well in a mixture of alcohol and water, in which it is completely insoluble. Likewise lipase, though not soluble in water as an emulsion-colloid, is capable of hydrolysing fat distributed in water. It follows then, that in every fluid in which an enzyme is at work, at least

two phases have to be distinguished. Now considering that of the particles of the enzyme only the surface comes into contact with the matter on which it can act, the action of the enzyme must necessarily be affected by the forms of energy, such as surface tension and electric charge, operating between every particle and the fluid in which it floats.

If a substance that is being dissolved in a fluid, lowers its surface-tension, that substance will accumulate above all on the surface, which is in contact with the air. Thus it is assumed, that canesugar, for instance, will also be accumulated, adsorbed, on the surface of particles of invertin, floating in the solution, so that the enzyme has the disposal of a much larger number of sugarmolecules to hydrolyse them to glucose and fructose. Bayliss also demonstrated experimentally that substances which lower the surface tension more than the substance does which is to be attacked by an enzyme when they coincide in the fluid, retard the enzymic action. Carbon adsorbs saponin much more intensely than ureum, in connection with the greater power of saponin to lower the surface tension. When saponin was added to a ureum-solution, less ureum was adsorbed from this solution by carbon than from a pure solution of that substance. In perfect accordance with this also the action of urease on ureum was inhibited by the addition of saponin, though this substance left the ureum itself unaltered. The reaction of the enzyme on the ureum was retarded, because the enzyme could not accumulate so much ureum in its immediate proximity, as the ureum was partly repulsed by the saponin, but the reaction was not arrested: after some time the same equilibrium was reached as in the pure ureum-solution, for the rest under similar circumstances [1]).

[1]) Arch. neerl. de Physiol. T. II p. 621.

What has been said above enables us to form some idea about the way in which the enzyme accelerates the reaction. This also makes it easy to understand, that the velocity of the conversion increases with the quantity of the enzyme, but that the total amount of matter that can be converted by the enzyme, is independent from the quantity of the enzyme. For, according as the number of enzyme particles and consequently their total surface is greater, a larger number of molecules of the matter adsorbed to the surface, will be converted in the same time; if, however, the solution contains but little enzyme, its particles will, unless they are destroyed by casual circumstances, be able to adsorb fresh molecules again and again, because the hydrolytic products are diffused in the neighbourhood; consequently as much of the substrate can be decomposed as corresponds with the equilibrium between substrate and splitting products, though it takes up more time.

— In many cases, however, also the substance to be decomposed, the substrate, belongs to the colloidal substances. It will hardly do to apply, without further consideration, what holds for dissolved substances with regard to the influence on the surface tension to colloidal substances that are not actually dissolved but are suspended in the fluid, to the influence on the surface tension. Meanwhile we should consider that there is no distinct boundary line between truly and colloidally dissolved substances and that, especially of the hydrophilous colloids such as protein and allied substances, a small part moves about as free molecules in true solution. Experience has taught that hydrophilous colloids may spread through diffusion, may cause osmotic pressure and lower the surface-tension. The assumption is warrantable, therefore, that they also are aggregated on the surface of the enzyme-particles.

But it can hardly be believed that with colloidal substances the quantity of enzymes adsorbed, in consequence of the surface tension, could be as large as with crystalloidal matter. Here the electric charge of the colloidal particles may play a part. A negatively charged particle of pepsin e. g. can adsorb protein particles that are positively charged in consequence of the acid reaction of the fluid, to that here also the concentration of the substance to be decomposed, is very considerable on the surface of the enzyme.

That enzymes and other colloidal substances can indeed be closely combined, has been proved in several cases. Many years ago already Von Wittich found that fibrin, merged in gastric juice that has been cooled down, consequently at a temperature at which only very little of the fibrin is splitted up into albumoses, combines with pepsin, which combination is not decomposed by washing in water. When the washed fibrin is warmed to bodytemperature, in pure dilute hydrochloric acid, it is soon dissolved and digested, just like unchanged fibrin in gastric juice. The same phenomenon may be observed in detail in the following way. To a solution of pure pepsin in 0.2 % HCl a small quantity is added of a solution of salt-poor protein, albumin or casein, in hydrochloric acid of the same strength. A precipitate is then yielded that, after being filtered, washed in cold hydrochloric acid and subsequently warmed in hydrochloric acid to bodytemperature, will soon be dissolved and split up into albumoses. — In an excess of protein the first precipitate is dissolved again. The pepsin is charged negatively, the protein has a positive charge under the influence of the acid. They combine and will then, if not too much protein is added, form an iso-electric complex, which collects in coarse flakes and is thrown down. With an excess of protein,

however, the complexes are positively charged and are diffused again through the fluid.

Compounds have also been made of starch and amylase, of trypsin and casein, in which the enzyme digested the adsorbed substance, not, however, before the conditions were made favourable to this process; but also adsorption compounds have been prepared of enzymes and substances they can not attack, of trypsin and starch, of amylase and casein. This need not surprise as also finely diffused carbon is capable of removing numerous enzymes from the fluids in which they are present.

The adsorption by the enzyme of the substrate to be digested, seems to be the first step to digestion, which may be compared to putting fuel in the hearth. Something else is required to effect combustion — the fire much be kindled. In the same way it will not suffice, that the particles of the enzyme gather the particles that are to be decomposed closely round them — they must exert an influence in another way also.

With respect to carbohydrates Emil Fischer has shown that the capability of enzymes to attack these substances is determined by their configuration [1]). He showed that maltase from beer-yeast is capable of hydrolysing the compounds derived from α-glucose, but not those derived from β-glucose, whereas with the β-glucosidase occurring in bitter almonds just the reverse happens, as mentioned above in connection with Bourquelot's researches on synthesis by means of enzymes.

Furthermore it appeared that several polysaccharids, varying only in configuration, require definite various enzymes in order to be hydrolysed. This induced Fischer to compare an enzyme to a key that is capable of

---

[1]) Ber. Deutsch. Chem. Ges. XXVII S. 2985 and Zeitschr. f. physiol. Chemie Bd. XXXVI S. 60.

acting only on a substance to which its fits as to a lock.

Now this comparison involves the idea of a true chemical compound, in contradistinction to an adsorption-combination. For in the latter case now a larger, now a smaller number of particles are aggregated on the surface of the enzyme, whereas in the former the molecules of the substrate to be decomposed, are combined with the molecules of the enzyme, in a manner and in a number that depends on the structure and the configuration of the enzyme and of the substrate.

The manner in which such a combination would be able to decompose or to compose the substrate can, for the present, be only vaguely imagined. It might be imagined, for instance that a molecule of α-methyl-glucosid attached to maltase, easily breaks off at the junction of glucose and methylrest, while by this process the glucose itself also is detached from the enzyme, so that by the aid of water, methylalcohol and glucose are set free. If glucose and methylalcohol are present in excess, the glucose may be attached to the enzyme — it should be remembered that it concerns an equilibrium-reaction — subsequently to the alcohol and ultimately be liberated again as glucosid. The action of the enzyme would then amount to this that, when attacking the glucose it affords greater mobility to the junction between glucose and alcohol, while on the other hand the glucose itself is easily detached directly when it has been liberated from the alcohol.

— It is no longer contrary to the current opinions about the structure of the molecules to imagine that a chain like a glucosid, attached to an enzyme at one end and made to swing, breaks at a certain weak point. Nor does it seem unreasonable to imagine that a molecule attached to an enzyme on one side, may be moved vehemently, when we take into consideration that the

action of an enzyme is promoted by a rise of temperature in such a sense that this process is limited only by the power of resistance of the enzyme to heat. But suchlike considerations are profitable only when they lead to new researches. For the present they seem to be somewhat premature.

That every enzyme exerts its action only on certain groups of substances of a definite composition and configuration, holds good not only for the hydrolysis and the synthesis of carbohydrate compounds, though in this case it has manifested itself most distinctly. Lipases attack only esters of fatty acids, proteases, several varieties of which are known, can decompose only proteins, or at all events complexes of amino-acids. This also suggests a chemical combination, in fixed ratios of weight, of the enzyme and the substrate. This view is favoured also by Ringer's [1]) experience that, as appeared from an investigation in the electric field, pepsin in acid solution combines with proteins and albumoses, not, however, with amino-acids, which it does not attack; if we had to do here only with an adsorption compound, it might also be expected with amino-acids, which in an acid solution are positively charged, just as protein and albumoses, whereas pepsin has a negative charge. The key of the pepsin fits in the lock of the protein, not in that of the separate amino-acids that are detached from it.

The great difficulty that presents itself again and again in the investigation of enzymes is the insufficiency of our knowledge of their nature. They are colloidal substances, occurring in fluids or in tissues of animals and plants, and mixed with a number of other substances, which they grasp tightly by adsorption, while, in endeavouring to

[1]) Onderz. Physiol. lab. Utrecht 5e R. XVI p. 252 and Zeitschr. f. physiol Chemie Bd. XCV, S. 195.

purify the enzymes, they are so easily changed — through modification of the reaction, through raising the temperature, or through influences of another nature, — that in the purifying process the enzyme is liable to be destroyed. The failures in endeavouring to prepare enzymes of some purity have been so numerous, that some profess it to be impossible, or have even expressed their opinion that enzymes are not peculiar substances, but substances being in a peculiar condition, much like that of iron in the magnetic condition.

Conceptions of such a nature, barring all further research, seem hardly justifiable. As regards the purifying of some enzymes, there has been made progress so far at all events, that we are no longer quite in the dark about the nature of the substance.

In this connection I feel urged to call attention to the enzyme, whose presence in the gastric juice had already been surmised by Van Helmont, viz. pepsin which is capable of hydrolysing protein with an acid reaction.

— The dog's gastric juice, an ample store of which may be obtained after a method suggested by Pawlow, without any pollution ensuing from substances emanating from the mouth or the intestine, acts powerfully on protein and is consequently rich in pepsin. Here we have a very favourable opportunity to free the enzyme from impurities, first of all because the gastric juice contains but few other substances and there is consequently relatively small chance for the enzyme separated from it to be considerably contaminated by adsorption, and secondly because the pepsin it contains, can be thrown down by substances that do not alter the enzyme, and unto which, as to solubility, pepsin behaves differently from most other substances. Pepsin namely is soluble, above all at body-temperature, in hydrochlorid acid of about 0.2 %, much less in acid of about $^1/_{10}$ of that percentage, readily soluble

again in water. When therefore gastric juice, which is a clear fluid, is dialysed against distilled water, it soon gets cloudy, and when the wished for acidity is reached a deposit is thrown down, that is easy to remove from the fluid. This deposit still holds a slight quantity of a colloidal substance not belonging to the enzyme, and distinguished from it i. a. by a much smaller degree of solubility, also at body-temperature, in 0.2 % HCl. Not only this circumstance may be utilised, also the property of pepsin to be completely thrown down the moment when the solution is saturated for one half with ammoniumsulfate, in which process a number of other substances, e. g. albumoses, remain in solution. It is not necessary to describe the preparation in full. The result is that a colourless substance is obtained, that displays the same very peculiar properties in various preparations. It is in complete accordance with proteins as regards reactions and the elementary composition, while it does not contain phosphorus. As an enzyme it acts very powerfully: 0.001 mgr. is able to dissolve 100 mgr. of dried and finely rubbed carminfibrin in 10 cc. of 0.1 % HCl at roomtemperature in the time of 18 hours. A substance possessing the same properties and an equally powerful proteolytic activity, may be prepared from the pig's gastric mucous membrane although in this case the cleansing cannot be performed so well, in consequence of the large quantity of all sorts of admixtures present in the extract of the mucous membrane.

The supposition that the enzyme, the pepsin, is not this protein itself but an admixture of it, is highly debatable. First and foremost it is already difficult to assume that the enzyme should be only an admixture of the protein, if we consider that an extremely small quantity of this substance is still capable to evince very distinct activity and that the activity of various preparations either from the dogs gastric juice, or the pig's gastric mucous membrane

is very constant. It is hard to believe that an admixture would contaminate the protein always in precisely the same degree.

It is also noteworthy that the substance, warmed in acid solution loses its activity as a pepsin, just at that temperature at which the protein is decomposed. Besides in this process it is proved in another way, that in different preparations the quantity of the enzyme agrees with that of the protein. The decomposition of the protein, namely, varies with the rate of heating. Slow heating keeps the fluid clear, rapid heating evolves a flaky deposit. Now the quantity of this deposit is proportional to the quantity of the enzyme present in the solution prior to heating.

If the enzyme were mixed with the protein only, perhaps bound to it by adsorption, such proportionality could hardly be expected. Thus it is, that also in the enzyme prepared from the pig's gastric mucous membrane, which can never be completely deprived of phosphorus-containing substances, now more now less phosphorus is found. I, therefore, believe that we are forced to suppose that this very peculiar protein molecule carries certain groups of atoms, by which it is enabled to take up other protein molecules and then make them crumble to pieces. It might be supposed that such groups of atoms could be detached from the protein and prepared independently just like the nucleic-acids of the nucleoproteids, though it is very doubtful whether with a similar hydrolysis, the activity, which in the case of enzymes is little proof against chemical proceedings, should not get lost. If so, even though the separation were effected, it would not be possible to recognise those groups of atoms because their only distinguishing feature is their very activity as an enzyme.

Indeed the protein of the pepsin in albumoses can be made to break up by digesting the purified substance in

hydrochloric acid at body-temperature. If this takes place in a dialyser that has been placed in hydrochloric acid of the same strength, the evolved albumose penetrates through the wall of the dialyser, while in the dialyser the enzyme disappears in proportion to the amount of solid matter. The pepsin has digested itself. Neither outside the dialyser nor inside it the enzyme is any more to be found.

This experience also tends to show that the enzyme constitutes a part of the protein-molecule. For if the pepsin were a mixture of protein and enzyme, the protein would be split up into albumoses and the enzyme would remain over, while in this case the enzyme itself disappears according as the protein is broken up.

— The properties of proteins may undergo various significant alterations owing to the annexion of so-called prosthetic groups. Nucleoproteids, haemoglobin, e. g. are dextrorotatory, whereas protein itself is levorotatory. Pepsin is levorotatory, but differs in other respects from proteins, e. g. in not being an amphoteric substance, but one that in acid solution, independent from the acid content, is always negatively charged. When dissolved in hydrochlorid acid a great part of it is decomposed in an electric field of high tension, the liberated protein is then transferred to the negative pole, but all that remains of the enzyme arrives at the positive pole and exhibits not only proteolytic activity but also invariably protein-reactions.

Still another difference between pepsin and other proteins was dicovered by Ringer, viz. that pepsin can combine a little more of an anion, Cl, in relation to H-ions [1]). There is no reason for assuming that this difference cannot be dependent on a prosthetic group.

It seems to me, therefore, that the nature of pepsin is

---

[1]) Onderz. Physiol. lab. Utrecht, 5e R. XVI p. 252 and Zeitschr. für physiol. Chemie, Bd. XCV, S. 195.

no longer shrouded in obscurity so much, as some are still thinking. True, our knowledge of it is very limited, which is not surprising, if we consider that the purification of pepsin presents many difficulties. Up to now only few workers have undertaken it. Still, the pepsin separated from the gastric juice, furnishes at all events material to proceed with the inquiries.

Another instance of an enzyme about whose nature we have to say something, is thrombin, the enzyme that effects the transition from fibrinogenous matter to fibrin. It is found in bloodserum. When serum is mixed with an excess of alcohol, a precipitate is formed, that is chiefly composed of proteins, and that, after being dried, is deprived by water of thrombin which was called originally fibrinferment. The solution thus obtained, which contains but very little solid matter, excites clotting when to an adequate amount it is mixed with a fibrinogen solution prepared from bloodplasm: the fibrinogen, a protein of the class of globulins, is converted into a solid gel. According to Hekma[1]) this change is reversible. The gel, called fibrin can be thrown into solution again by the aid of very dilute sodiumcarbonate. This solution may exhibit some analogy to a solution of fibrinogen, but I think Hekma is wrong in identifying the two. In the fibrin-solution a jelly-like precipitate is evolved by the addition of some drops of 1 % $CaCl_2$, whereas under the same treatment a fibrinogen solution undergoes no visible change. Nor has thrombin, which causes a fibrinogen solution to coagulate, the slightest effect on a fibrin solution, unless the thrombin be mixed with limesalt, which is alone sufficient to form a jelly-like precipitate. The conclusion is, as I think, warrantable that fibrinogen,

[1]) Ned. Tijdschr. v. Geneesk. 1916 II p. 831. More detailed in Biochem. Zeitschr. 1916.

in being converted into fibrin, undergoes not only a physical but also a chemical change, as is generally the case in coagulation of proteins.

A. Schmidt, who was the first to prepare thrombin from bloodserum, came to the conclusion that this substance must be regarded as an enzyme, because small quantities can coagulate a large quantity of fibrinogen just as well as large ones, because after the completion of the coagulation, the fluid still contains thrombin, which gradually decreases in quantity only after renewed action, and because the substance acts best at bodytemperature and is destroyed by heating to 65° C.

Later on this point has been discussed by Rettger [1]), whose work in this direction is considered by Bayliss [2]) of much importance. But in the majority of cases his experiments have been described so incompletely as to render it difficult to assign to them any conclusive force of argument in the presence of numerous observations of other workers. Rettger's principal argument against the hypothesis that thrombin is to be regarded as an enzyme, is his experience that a solution of fibrinogen yields more fibrin in presence of a large quantity of thrombin than a small one. This would hold only, if besides thrombin also limesalt were present in the fluid, a circumstance that has not been investigated by Rettger. It is well known that thrombin, thoroughly purified, that does not contain free limesalt, is capable of converting only a definite amount of fibrinogen into fibrin, but is activated anew as soon as a little limesalt is added [3]). Since Rettger has not reckoned with this circumstance,

---

[1]) Amer. Journ. of Physiol. Vol. XXIV p. 406.

[2]) The Nature of Enzyme action. London 1914 p. 96, 143.

[3]) Onderz. Physiol. Lab. Utrecht 4e R. II p. 41 and Verh. Kon. Akad. v. Wet. Amsterdam, Unters. über Fibrinferment 1892 S. 30.

his figures do not prove anything for a limited activity of thrombin. Furthermore Rettger assures us that thrombin is proof against heating, nay even boilingheat, without exactly describing any experiment to substantiate his hypothesis — and this might reasonably be expected where it regards a pronouncement at variance with the experience of a large number of other observers. More valid arguments are wanted in contesting the statement that thrombin must be reckoned among enzymes.

Blood-plasma protected from coagulation in one way or other, does not contain thrombin. It does contain, however, a mother-substance of it that by the addition of limesalt, is converted into thrombin. From such a plasma may be prepared a nucleo-proteid that can be converted into thrombin, with the aid of lime. Besides by Schmidt's method the enzyme can be liberated also with the aid of acetic acid from blood-serum that contains thrombin. In either case it can be proved that the enzyme consists chiefly of a nucleoproteid, that contains lime, but in such a sense that the lime cannot be precipitated from it by an oxalate, in other words that it is not in an ionised condition. The same is the case with the nucleoproteid prepared from plasma and then combined with lime.

Not only nucleoproteids of the bloodplasma, but also those prepared from all sorts of organs, thymus, liver, kidney, testis, muscles, spleen, glandula submaxillaris are capable of coagulating, as soon as they combine with lime, a fibrinogen solution in quite the same way as the thrombin of the bloodserum. Not all workers have been in a position to confirm this experience. This proved to be due to the fact that they did not purify the nucleoproteids and the fibrinogenous matter as well as could be, but used for their experiments extracts of organs and bloodplasma, fluids contraining a number of other substances, which could inhibit or even prevent the formation of fibrin.

The nucleoproteids from which the thrombin is evolved that makes the blood coagulate, when it is drawn from the body, are produced by the corpuscles floating in the blood, in the first place by the blood-platelets, which are injured on issuing from the vessels. Then the nucleoproteid in combination with the limesalts in the blood, forms thrombin.

On injection of not too small a quantity of nucleoproteid, into the blood of a healthy animal, notably a rabbit, will evolve, with the aid of limesalts present in the blood, thrombin and consequently fibrin in the flowing blood by which the blood-vessels are obstructed. In this case also, as Halliburton has shown, nucleoproteids of various origin act similarly; they all form thrombin with the limesalts dissolved in the blood. No doubt also under normal conditions nucleoproteids, originating from injured blood-corpuscles, are thrown into solution. In small quantities, however, their obnoxious influence can be neutralised in the body. They are decomposed, whereby albumoses appear as decomposition products. It follows that then the nucleoproteids are prevented from developing thrombin.

There is often a tendency to assume that thrombin does not take its origin in the nucleoproteids themselves, but that the enzyme is to be looked for in admixtures, still unknown; a hypothesis that cannot be refuted conclusively until researchers have succeeded in preparing chemically pure nucleoproteids, without any denaturation. However this supposition must then give rise to new hypotheses, that can hardly be deemed acceptable. The nucleoproteids prepared from the animal body or from the blood are substances easily modificated. They do not resist acids very well and are still more sensitive to alkalis; even in a neutral solution standing gradually lessens the solubility, so that the fluid gets turbid. At the same time the power to form thrombin with calcium decreases. We should,

therefore, have to assume that the supposed admixture was changed under precisely the same circumstances as the nucleoproteid. Thus it is also with the action of heating. From calf's thymus two kinds of nucleoproteids were prepared, that were denaturated through heating at a different temperature. From both thrombin was formed by the addition of $CaCl_2$. The solution of the one substance was rendered inert through heating for 15 min. at 54° C., while the other had to be heated for the same lenght of time at 60° C. to lose the power of coagulating fibrinogen with $CaCl_2$. Both nucleoproteids had been prepared from the same organ. If the activity had been conferred by an admixture, it would of necessity be of the same nature in either case. We observed, however, that the enzyme action was lost first by the nucleoproteid that was first denaturated through heating. Moreover the quantity of a nucleoproteid, required to evolve coagulation, particularly when the substance is thoroughly purified, is very small. Nucleoproteids from thymus, fitter than most others for purifying, caused complete coagulation in a fibrinogen solution, when the coagulation-mixture contained 0,05 mgr. of it to 1 cc. [1]).

In spite of all the different conceptions of the nature and the origin of thrombin advocated by various writers, it seems to me, as I have tried to show in various papers, each time on the basis of fresh observations, that we are justified in regarding thrombin as an enzyme, formed by the combination of some nucleoproteid with calcium.

Here then we are confronted with a considerable difference from pepsin, an enzyme of very specific composition, that so far as we know now, is in every respect of the same nature in various mammals and is capable of splitting, with acid reaction, all sorts of proteins of varying

---

[1]) Onderz. Physiol. Lab. Utrecht, 5e R., IV, p. 451 and Zeitschr. f. Physiol. Chem. Bd. XXXIX, S. 22.

properties and composition. Thrombin, on the other hand, exhibits activity exclusively on a single protein, a globulin of specific nature, the fibrinogenous substance of the blood. On the other hand it differs from pepsin in being varying in composition since it can be formed from all kinds of nucleoproteids, if not denaturated.

Nevertheless, however different they may be, pepsin and thrombin, as enzymes, can be brought under the same point of view, when it may be taken for granted that the action of the enzyme is bound up with one or perhaps more specific groups of atoms, that constitute only a small part of the whole, no doubt very large, protein molecule. Now the nucleoproteids also contain specific groups of atoms, nucleinic acids all decidedly of the same nature, however much they may differ from each other as to their composition. In this respect they all agree, and this may be the cause, that they are all capable, when combined with calcium, of converting fibrinogen into fibrin.

In the instances described above the groups of atoms, whose activity depends on the enzyme constitute a part of a protein molecule.

It has been said more than once, that enzymes cannot be proteins, because enzyme solutions, that work well, do not always exhibit protein reactions. This pronouncement, however, is not well founded, especially not for pepsin, as may be proved. When the purified substance, that gives all the protein reactions, is dissolved in hydrochloric acid, and the solution is diluted with hydrochloric acid, the reactions get gradually fainter, but the one sooner than the other; for example the xanthoprotein-reaction is still perceptible after Millon's reaction has disappeared. Nobody will presume that when the latter reaction is no longer visible, the fluid does not contain a protein with a tyrosin-group. After the dilution also the proteolytic action

decreases, but it is still distinctly perceptible after also the xanthoprotein reaction has entirely disappeared. It is still very distinct when the pepsin content of the fluid has fallen to 0.00005 % or even lower.

What reason is there for thinking that this reaction does not depend on the protein dissolved in hydrochloric acid?

— However, fom all this it does not follow that all enzymes are proteins. It is indeed possible to prepare invertin that contains hardly any nitrogen and that — which in saying a great deal — acts more intensely according as the nitrogen content decreases. When thinking of the decisive influence of the structure of a carbohydrate molecule on the possibility of a certain enzyme to attack it, it would not seem at all surprising, if the activity of such an enzyme was depending on carbohydrates of a certain configuration, whether or no constituting a part of larger complexes, in the manner of prosthetic groups.

A comparatively simple structure seems conceivable of enzymes that, in normal cases, are driven out by the cells and display their activity only then, as may be observed with the enzymes occuring in the alimentary canal. In the cells of the glands of the stomach, of the pancreas, substances are formed, deposited in the shape of granules or droplets in the protoplasm, and act as enzymes only after they have been secreted and have reached the alimentary canal.

There is, however, another group of enzymes, the so called intracellular or endo-enzymes, that do not leave the cell during its life. Sheridan Lea was the first to furnish a specimen in connection with bacteria capable of changing ureum into ammoniumcarbonate [1]). Musculus had shown that from similar bacteria, when dried and

[1]) Journal of Physiol. Vol. VI p. 136 1885.

treated with alcohol, an enzyme could be obtained by extracting with water, that acted very powerfully in converting ureum. Lea now found, in experimenting on urine, in which similar bacteria had been largely developed and had brought about a strong ammoniacal fermentation, that the fluid, filtered through some layers of filterpaper, so that in the filtrate no bacteria could any more be detected under the microscope, did not contain anything of the enzyme, but that from the filtered bacteria, after being killed with the aid of alcohol, the enzyme could be prepared in large amount, through extracting with water. It was only some years later that the appearance of endo-enzymes attracted more attention, notably when Buchner had made out that injured yeast-cells impart to their environment an enzyme that in normal life they obstinately cling to, viz. zymase, which evolves alcohol and carbonic acid from sugar.

— Beyerinck has assigned a general and a great significance to these endo-enzymes. In his "Enzymtheorie van de Erfelijkheid" [1]) he has advanced the hypothesis that the particles of the protoplasm that are most often called gens or pangens, and are held to be the carriers of hereditary qualities, are to be looked upon as enzymes. He believes, therefore, that hereditary qualities such as the power to generate light or colouring matter, must be ascribed to endo-enzymes. In the same way the formation of the cellwall rests on inherited enzymes in the cell, which Beyerinck has illustrated by a large number of examples borrowed from microbes.

— According to this theory, which opens up fresh points of departure in the science of heredity and in the nature of the protoplasm, endo-enzymes in the uninjured cell belong to living matter: they possess its most charac-

---

[1]) Versl. Kon. Akad. v. Wet. Amsterdam Dl. XXV, p. 1221, 1917.

teristic quality, the power of assimilating the comparatively simple constituents of food, i. e. of building up from it new living matter and of multiplying. Once detached from the cell, they have lost this power, but if not injured otherwise, they retain the power to exert, as enzymes, an influence upon chemical changes of certain substances, with which they come into contact. It can hardly be doubted that these endo-enzymes, fragments of the living matter, must be of a highly developed composition and will certainly also contain protein within their molecule.

From whatever side we approach the subject of enzymes, we are still confronted with riddles, for whose solution we only strive with the aid of conceptions, and hypotheses at best probable, not at all proven. Nevertheless, conceptions, hypotheses, theories are essential to guide us in further investigation. Considering how little was known about enzymes half a century ago, and comparing it with what we now profess to know in virtue of manifold, repeated observation, we may hopefully anticipate a wider view of and a deeper insight into enzymes, resulting from a continued, many-sided inquiry, which, though guided by hypotheses, should not on that account be prejudiced.